REMARKS

Amendments to the Claims

Applicant has amended Claims 23, 25-28, 59, 60, 62. New Claims 66-68 have been added. Support for these amendments and claims is found throughout the specification. For example, support for allele frequencies determined relative to a specific population is found on page 17, lines 13-19, and page 21, line 29 through page 21, line 1. Amended Claims 26-28, 59 and new Claims 66-67 use the term "nonsynonomous mutations". The terms "nonsynonomous" and "synonomous" are standard terms of art used to describe types of mutations. "Synonomous" mutations are mutations in the genetic code that cause amino acid mutations where the "new" amino acid has the same or similar properties as the original with no change in the conformation or characteristics of the protein. A synonomous mutation is often referred to as a silent mutation. "Nonsynonomous" mutations produce a different amino acid resulting in a modified protein with changes in the conformation or characteristics. (See, e.g., http:www.evolutionpages.com/mouse%20genome%20genes.htm; http:www.science.mcmaster.ca/biology/CBCN/genetics/hud-gloss.htm). The specification describes mutations on page 25, lines 7-18; page 36, lines 12-21; page 37, lines 15-21; page 42, lines 16-23 and throughout the specification. Mutations are described as frameshifts, stop codons, splice site changes, inactivating missense mutations, additions, deletions, silent and crytic. Thus, synonomous and nonsynonomous mutations are described in the specification. No new matter has been added. Entry of these amendments and new claims is respectfully requested.

Rejection of Claims 25-28, 33, 59 and 60 Under 35 U.S.C. 112, First Paragraph

The Examiner has rejected Claims 25-28, 33, 59 and 60 under 35 U.S.C. 112, for failing to comply with the enablement requirement.

Applicant points out that the Specification details a method for detecting mutant fragments obtained from one or more samples, wherein mutant fractions are enriched, amplified by HiFi-PCR, and then, for example, isolated by CDCE (pages 17-21). Khrapko *et al.* teach that this combination of methods allows the detection of point mutations in biological samples at or above 10^{-6}. Therefore, in combination with what was known in the art at the time of filing, Applicant discloses a method useful for identifying genes with harmful alleles based on comparing the sum of allele frequencies. Obtaining statistically relevant comparisons, a large number of mutations are sampled, thereby

requiring a method for detecting point mutations occurring somewhat infrequently, *e.g.*, a method capable of detecting point mutations at or above a frequency of 10^{-6}. Applicant points out, however, that this does not ***necessarily*** mean that all identified point mutations will occur at such a low frequency. The Examples the Examiner refers to indeed indicate that identified mutations can occur at a much higher frequency, orders of magnitude higher, for example. However, the ability to determine frequencies for many alleles of a gene is necessarily dependent on the sensitivity of the detection method and ability of the method to determine frequencies of relatively rare alleles as well as more commonly occurring alleles. A screen for point mutations that samples all mutations with a frequency at or above 10^{-5} provides more complete data than a screen that can only detect mutations occurring at a frequency of, for example, 10^{-1}. Therefore, Applicant asserts, that there is abundant support for the mechanical methods for detecting such point mutations in samples, as methods are described that can detect relatively rare alleles. Therefore, the specification provides guidance, for example, as to populations to be screened and how they are to be screened. One of skill in the art would know that the mechanical process of screening itself can be performed by means such as, for example, CDCE plus HiFi PCR, which the art shows can detect mutations well within the range of the claimed invention.

Guidance in the Specification

The Specification describes the combination of HiFi PCR with a number of techniques for isolating heteroduplexed DNA (see, for example, Page 18, line 21 through page 21, line 5). This enabling guidance would allow one of skill in the art to detect, in a sample, all point mutations that occur at an allele frequency of 10^{-4} in a population from which the sample was obtained. Therefore, given the fact that the combination of HiFi PCR and CDCE can detect mutations at frequencies as low as 10^{-6}, the guidance in the specification is clearly sufficient to enable the breadth of the claimed invention.

Unpredictability and State of the Prior Art

Applicant has amended the claims to delete references to "inherited" mutations. The Examiner points out that even with such detection techniques, the claimed invention is not enabled for the full scope of detecting all inherited point mutations of a gene at a frequence at or above 10^{-5} because of population size and idiosyncracies. Applicant has amended the claims to reflect that the

allele frequency of the point mutations is relative to a specific population from which the sample was obtained, thereby obviating the rejection. For the purpose of identifying a gene carrying harmful alleles, it does not matter that possible or rare alleles are missed. The determination is made on the basis of the total number of mutant allelic copies, the total number of nonsynonomous allelic copies or the total number of obligatory knockout mutation copies in the actual sample, non in the entire population sampled, e.g. the United States. If both the populations of young and aged individuals comprise a similar genetic background or mixtures of genetic backgrounds as in the United States, then differences in frequencies of alleles associated with a particular gene will identify the particular gene as having harmful alleles. At the sample sizes necessary to detect significant differences between young and aged populations with regard to the number of allelic copies that encode a mortal disease the sampling variation for major ethnic groups such as European, African and Asian Americans is not expected to interfere with detection of genes carrying alleles for common mortal diseases that afflict all such groups. If there is no difference, then the method of the present invention will not identify it as such. Applicant notes that the method of the invention is directed to identifying genes that carry harmful alleles in the sense as encoding risk for a common mortal disease; the invention is not necessarily directed to identifying all possible alleles of any particular gene.

Applicant further points out that the state of the art with respect to detection techniques, *e.g.*, HiFi PCR and CDCE, was demonstrated to be predictable, as discussed above, in the sense that detection limits and methods were known to one of skill in the art.

Quantity of Experimentation

The Examiner points out that the steps required for a screen according to the scope of the claimed invention would constitute undue experimentation. The Examiner's assertion that every point mutation in a gene in a population would need to be identified is not a correct characterization. The method of the present invention allows for the sampling of each member of a population, and, therefore, all point mutations capable of being detected (*e.g.*, above a frequency of 10^{-4}) are screened, within the chosen population. Applicant respectfully submits that this is a significant advantage to Applicant's claimed invention in that it provides for rapid and comprehensive screening methods that significantly overcome laborious iterative methods, such as, for example, those taught by Kervinen *et al.* (see below). Current methods tend to look for harmful alleles where

a single harmful allele is responsible for the disease, and therefore are limited to identifying harmful alleles only where it is the case that a single allele is responsible for the disease (e.g., cystic fibrosis). However, the failure of such methods for common diseases rests in the fact that there are often several alleles that are causative where no one single allele rises to the level of being detectable in current labor-intensive searches. Applicant's invention utilizes a different approach in the all mutations in a population, occurring at a frequency about a particular threshold determined by the method of detection, are determined in, for example, young and old population. Genes with a significant difference in sum frequencies due to allele frequencies of alleles associated with, for example, mortal disease, will be identified. Therefore, it is the gene that is identified as having multiple alleles associated with, for example, mortal disease. This method is not dependent on genetic differences between ethnic populations, although selecting young and old populations from the same genetic background would reduce noise associated with non-harmful alleles.

The situation is analogous to the genetic distinction between selecting and screening. The "undue experimentation" the Examiner refers to is a "screening"-type method, where each point mutation would have to first be identified and its frequency determined. Such a method would indeed be very labor intensive. However, the "selection"-type method of the claimed invention samples all mutations in a population above a frequency determined by the limit of detection of the detection technique. Applicant's invention pertains to methods where all mutations are scanned for all genes in young and old populations, and genes with statistically significant sum frequencies are identified as, for example, harmful.

In light of the above remarks and Applicant's amendments to the Claims, reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claim 59 Under 35 U.S.C. 112, Second Paragraph

The Examiner has rejected Claim 59 under 35 U.S.C. 112, as being indefinite for failing to particularly point out and distinctly claim that which Applicant regards as the invention.

Applicant has amended Claim 59, thereby obviating the rejection.

Reconsideration and withdrawal of the rejection are respectfully requested.

Claim Interpretation

Point (B). Applicant disagrees with the Examiner's assessment that because "inactive" was not defined in the specification, "any point mutations within a gene are considered as obligatory knock-out mutations, since potentially they can change protein activity." Applicant states that a change in activity is not equivalent to inactivity. In ordinary usage, "inactive" is not being active. Therefore, obligatory knockouts are, at least, mutations that cause a gene to not be active. A mere change in protein sequence does not lead to inactivity necessarily. Additionally, not all mutations in a coding sequence will lead to a change in the protein sequence, *e.g.*, silent mutations. The Examiner's statement that "any point mutations within a gene...can change protein activity" is not correct. Only specific point mutations within a gene can change protein activity, and, further, only a subset of these mutations will cause the gene to be inactive.

Rejection of Claims 23 and 62 Under 35 U.S.C. 102(b)

The Examiner has rejected Claims 23 and 62 under 35 U.S.C. 102(b) as being anticipated by Kervinen *et al.* as evidenced by Margaglione *et al.* (Stroke, 29:399-403, 1998) and Paik *et al.* (PNAS, 82:3445-3449, 1985).

Applicant has amended Claims 23 and 62. The rejection with respect to Claim 23 is obviated by Applicant's amendment, as discussed above. With respect to Claim 62, Applicant disagrees that a knock-out mutation can be a mutation where activity is merely "altered" as opposed to inactivated, as one would commonly use the term. An inactive gene cannot be active.

The Examiner states that Margaglione *et al.* indicate the ε4 allele has a Cys->Arg substitution at position 112, however, it is still taught that this allele is an isoform of apoE and, therefore, functional. The Specification, on page 25, lines 10-12, states, "Obligatory knock-outs are point mutations which necessarily inactive the gene, such as point mutations which introduce stop codons or frame shifts in exons of protein encoding genes." A Cys-.Arg substitution is clearly not an introduction of a stop codon or representative of a frameshift mutation. There is no independent evidence that the product encoded by the ε4 allele is non-functional. In fact, the description of the E4 product as an "isoform" of apoE in the Kervinen *et al.* reference is indicative of the fact that the gene is functional. If the ε4 allele is a functional gene, then it cannot be a knock-out mutation. thereby obviating the rejection as discussed above. As amended, Kervinen *et al.* do not teach every limitation of the claimed invention and cannot, therefore, anticipate the claimed invention.

Submitted herewith is the Declaration Under 37 C.F.R. § 1.132 of William G. Thilly. In his Declaration, Dr. Thilly specifically discusses the features of the claimed invention as distinguished from the teachings of the cited art.

In light of the above amendments and statements of Dr. Thilly, reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

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